

### Claims

1. An essentially homogenous preparation of metal-binding human transferrin free of other human proteins.

5

2. The essentially homogenous preparation of metal-binding human transferrin of claim 1, wherein the human transferrin binds to a transferrin receptor.

10

3. An essentially homogenous preparation of iron-binding human serum transferrin free of other human serum proteins.

4. The essentially homogenous preparation of iron-binding human transferrin of claim 3, wherein the human transferrin binds to a transferrin receptor.

15

5. Human serum transferrin which binds a metal, essentially free of other serum proteins, produced by:

- a) culturing a eukaryotic cell transfected with an expression vector of claim 14 under conditions conducive to expression of the transferrin; and
- b) recovering the expressed transferrin.

20

6. A mutant human serum transferrin half-molecule comprising at least the metal-binding domain of a single lobe of transferrin, but not the metal-binding domain of the other lobe, the mutant having a stronger binding avidity for metal than the binding avidity of natural human serum transferrin.

25

7. A mutant transferrin half-molecule of claim 6, which has a stronger binding avidity for iron than natural human serum transferrin.

8. A mutant transferrin half-molecule of claim 7, comprising at least the metal-binding domain of a single lobe of transferrin wherein the lysine residue at position 206 of natural human serum transferrin is replaced with glutamine.

5 9. A mutant transferrin half-molecule of claim 7, comprising at least the metal-binding domain of a single lobe of transferrin wherein the lysine residue at position 206 of the amino acid sequence of natural human serum transferrin shown in SEQ ID NO:2 is replaced with glutamine.

10 10. A mutant transferrin half-molecule of claim 7, comprising at least the metal-binding domain of a single lobe of transferrin wherein the histidine residue at position 207 of natural human serum transferrin is replaced with glutamic acid.

11. A mutant transferrin half-molecule of claim 7, comprising at least the metal-binding  
15 domain of a single lobe of transferrin wherein the histidine residue at position 207 of the amino acid sequence of natural human serum transferrin shown in SEQ ID NO:2 is replaced with glutamic acid.

12. An essentially homogenous preparation of non-glycosylated metal-binding human  
20 transferrin free of other human proteins.

13. The essentially homogenous preparation of non-glycosylated metal-binding human transferrin of claim 12, wherein the metal is iron.

25 14. An eukaryotic expression vector, comprising a nucleic acid construct comprising nucleic acid encoding a transferrin or a transferrin half-molecule comprising at least the binding domain of a single lobe of transferrin linked to appropriate genetic regulatory elements for expression in an eukaryotic cell.

15. An eukaryotic expression of vector of claim 14, wherein the nucleic acid construct includes a nucleic acid encoding transferrin signal sequence linked to the nucleic acid encoding the transferrin or transferrin half-molecule.

5 16. An eukaryotic expression vector of claim 15, wherein the single lobe is the amino terminal lobe of human serum transferrin.

17. An eukaryotic expression vector of claim 15, wherein the single lobe is the carboxy terminal lobe of human serum transferrin.

10 18. An eukaryotic expression vector of claim 14, wherein the transferrin half-molecule contains a glutamine residue at position 206 in place of the lysine residue of natural transferrin.

B

15 19. An eukaryotic cell line transfected with the vector of claim 14.

20. A baby hamster kidney cell line transfected with the vector of claim 14.

21. A method of producing functionally active human transferrin, comprising:

- 20 a) culturing a eukaryotic cell transfected with an expression vector containing DNA encoding the transferrin, under conditions conducive to expression of transferrin; and
- b) recovering the expressed transferrin.

25 22. A method of claim 21, wherein the vector is the plasmid pNUT.

23. A method of claim 21, wherein the eukaryotic cell is a baby hamster kidney cell.

24. A method of producing a functionally active human serum transferrin, comprising:
- a) culturing a eukaryotic cell transfected with an expression vector comprising DNA encoding human serum transferrin, or a portion thereof, operably linked to an inducible promoter of transferrin;
  - b) inducing the promoter in order to induce expression of transferrin; and
  - c) recovering the expressed transferrin.

25. A method of claim 24, wherein the promoter is the zinc inducible metallothionein promoter.

26. A nonserum supplement for cell culture medium containing human serum ransferrin produced by a method of claim 24.

add  
B'

00511 0126100

## **RECOMBINANT TRANSFERRINS, TRANSFERRIN HALF-MOLECULES AND MUTANTS THEREOF**

Recombinant transferrin, non-glycosylated recombinant transferrin,  
5 transferrin half-molecules and mutant transferrins having altered metal-binding or other  
properties are described. The recombinant transferrin molecules are expressed in  
functional form by stable eukaryotic cell lines such as baby hamster kidney cells  
transformed with an expression vector encoding the recombinant molecule. The  
recombinant transferrins can be used in metal chelation therapy to bind and clear excess  
10 toxic metals in patients suffering from metal overloads or as tissue culture medium  
supplements or replacements.